AD_____

GRANT NUMBER DAMD17-96-1-6106

TITLE: Breast Cancer Following Hodgkins Disease: Risk Factors and Intervention

PRINCIPAL INVESTIGATOR: Leslie Robison, Ph.D.

CONTRACTING ORGANIZATION: University of Minnesota
Minneapolis, Minnesota 55415-1226

REPORT DATE: July 1997

TYPE OF REPORT: Annual

PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release; distribution unlimited

19970929 061

REPORT DOCUMENTATION PAGE

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE July 1997	3. REPORT TYPE AND DATES COVERED Annual (1 Jul 96 - 30 Jun 97)

4. TITLE AND SUBTITLE

Breast Cancer Following Hodgkins Disease: Risk Factors and Intervention

5. FUNDING NUMBERS

DAMD17-96-1-6106

6. AUTHOR(S)

Leslie Robison, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Minnesota
Minneapolis, Minnesota 55415-1226

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
504 Scott Street
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

We conducted a study to evaluate the role of genetic predisposition (as measured by family history of cancer) in the development of breast cancer among the Late Effect Study Group cohort of survivors of Hodgkin's disease in childhood. Of 17 women with secondary breast cancer identified in this cohort, 13 probands (76%) or their surviving next of kin were available for construction of pedigrees. The median age at diagnosis of Hodgkin's disease for these patients was 13 years and that for breast cancer was 34 years. Nineteen family members were reported to have experienced cancer among the 180 first- and second-degree relatives (total follow-up of 9,351 person-years). Overall, there was a significantly decreased risk of cancer among the family members (SIR=0.6, 95% CI, 0.4-0.9). Breast cancer was reported in three family members (median age at diagnosis, 59.5 years), and there was no excess of breast cancer when compared to the general population. In an expanded assessment of the 13 cases with breast cancer developing at a very young age following treatment for Hodgkin's disease, we failed to demonstrate any evidence of familial aggregation of cancer (breast or otherwise) among family members. The influence of biomarkers of genetic susceptibility, need to be explored, in order to identify high risk populations.

14. SUBJECT TERMS Breast Cancer			15. NUMBER OF PAGES 25
			16. PRICE CODE

DTIC QUALITY INSPECTED 4

17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

_____ Where copyrighted material is quoted, permission has been obtained to use such material.

_____ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

_____ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

_____ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

_____ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

_____ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

_____ In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

_____ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

_____
PI - Signature   Date

Table of Contents

1.0 INTRODUCTION

Analysis of a cohort of 1380 survivors of childhood Hodgkin's disease (HD) from the Late Effects Study Group (LESG) has shown a 75-fold increased risk of breast cancer compared with the general population, with the cumulative probability of developing breast cancer approaching 35% by 40 years of age among the female survivors of HD. The median age at diagnosis of breast cancer was 31.5 years (15.4 to 42 years) and the median latency was 19.3 years (2.4 to 28.5 years). We hypothesize that patients with HD who subsequently develop breast cancer have a genetic susceptibility to develop second neoplasms, specifically breast cancer. The purpose of this proposal is to identify a subpopulation among the survivors of HD, that is at an increased risk for developing breast cancer, and to institute intervention in the form of active screening and possibly chemoprevention. We plan to obtain and validate family histories of individuals with secondary breast cancer in order to quantitate the risk of breast cancer in the respective families. We also plan to identify somatic and/or germline mutations in candidate genes known to be associated with breast cancer, including p53, BRCA1 and ATM. In addition, we plan to institute a surveillance protocol in the HD patients identified to be at a high risk of developing secondary breast cancer (age between 10 and 16 years at time of diagnosis of HD, mantle radiation), to look at the efficacy of mammography as a screening tool in early detection of breast cancer and in reducing mortality. There will be ongoing surveillance and expansion of the original cohort to recruit more patients to the study.

1.1 SPECIFIC AIMS

Analysis of a cohort of 1380 survivors of childhood Hodgkin's disease (HD) from the Late Effects Study Group (LESG) has shown a 75-fold increased risk of secondary breast cancer compared with the general population (Bhatia et al, 1995). **We hypothesize that patients with HD who subsequently develop breast cancer have a genetic susceptibility to do so.** The goal of this proposal is to identify a subpopulation among survivors of HD, that is at an increased risk for developing breast cancer. We will use an established and active cohort of female survivors of HD, diagnosed between 1955 and 1986 at one of the participating institutions of the Late Effects Study Group (LESG) Appendix. Thus far, seventeen patients have been identified with secondary breast cancer in this cohort.

1.1.1 Specific Aim 1.
To obtain and validate family histories of individuals with secondary breast cancer following successful treatment of HD, in order to quantify the risk of breast cancer in the respective families.

1.1.2 Specific Aim 2.
To identify somatic and germline mutations in candidate genes known to be associated with both breast cancer and sensitivity to radiation-induced carcinogenesis.

i Tumor tissue (paraffin-embedded or frozen) will be obtained from the 17 patients with post-HD breast cancer. Tissue will be examined, using PCR-SSCP and immunochemistry, for somatic mutations in p53, a gene known to be involved in both radiation sensitivity and in the etiology of breast cancer. Additionally, in frozen samples where RNA is available, tumor will be screened for mutations in the gene ATM which is mutated in ataxia telangiectasia.

5

ii Samples of peripheral blood will be obtained from those patients with breast cancer who are known to be surviving (n=12), and will be examined using PCR-SSCP for germline mutations in p53, and by RT-PCR and SSCP for germline mutations in the gene ATM.

iii A recurring mutation in exon 20 of the gene BRCA1 has been described in families with breast cancer and HD. PCR-SSCP will be used to screen the study population for germline or somatic mutation of BRCA1 at this site.

iv Samples of peripheral blood will also be obtained from control HD patients who have not developed breast cancer. Controls will be matched with the breast cancer patients for age, length of follow-up and treatment course. These samples will also be studied using PCR-SSCP for germline mutations in p53 and BRCA1, and by RT-PCR and SSCP for mutations in AT*M*.

1.1.3 Specific Aim 3.
To maintain and expand the cohort of HD survivors under surveillance, in order to incorporate any newly diagnosed patients with breast cancer into the current studies.

2.0 BACKGROUND AND SIGNIFICANCE
2.1 Hodgkin's Disease
Over the last three decades there has been a marked improvement in survival, with five-year survival rates now approaching 90%.(1) Because of this improvement in survival,.long-term sequelae of HD and its treatment (specifically second neoplasms) are now being encountered (2-5) In contrast to the risk of treatment-related leukemia, which does not appear to extend beyond 10 years, the risk of developing a solid tumor continues beyond 15 years. This is the most important problem facing HD patients and their physicians today.

2.2 Breast Cancer Risk following Radiation
An increased risk of breast cancer has been observed among women exposed to radiation during atomic bomb explosions, repeated chest fluoroscopies, or treatment of postpartum mastitis and women with early-stage HD who receive mantle irradiation.(20-26) Common features of radiation-associated breast cancers are the importance of age at first exposure to radiation (puberty) and the long latency period (at least 10 years from exposure).(26,27)

2.3 Genetic Susceptibility to Breast Cancer
The development of breast cancer after exposure to ionizing radiation also may be influenced by genetic susceptibility.(28,29) Claus et al (31) provide age-specific estimates of risk for women by type of relative affected with breast cancer as well as by age at onset of the affected relative. These data can be used to estimate a woman's risk of breast cancer for the purpose of counseling and decisions regarding time and frequency of active screening, and, at the time of diagnosis of HD, in trying to modify therapy.

2.3.1 Tumor Suppressor Genes and Breast Cancer
The p53 gene functions as a tumor suppressor gene and regulates the cell-cycle response to DNA damage. Somatic mutations of this gene are extremely frequent in human cancer, including breast cancer and HD. In vitro data have shown that the p53 tumor suppressor gene is an important participant in the cellular response to ionizing radiation, with cells lacking p53 being unable to arrest the cell cycle to repair DNA damage or enter into apoptic cell death following irradiation. Importantly, transgenic mice that lack wild-type p53 have an increased susceptibility to radiation induced carcinogenesis.(34) This susceptibility to radiation-induced carcinogenesis is associated with a two-fold increase in the in vivo accumulation of radiation-induced double-stranded chromosomal breaks relative to those observed in wild-type animals. Thus, it appears that one of the ways in which p53 acts to suppress tumor formation in vivo is by preventing the accumulation of

cells that have sustained radiation-induced DNA damage. These data indicate that germline mutations in p53 present in patients with Hodgkin's disease would predispose those patients to radiation induced second malignancy, including breast cancer.

2.3.2 Ataxia-Telangiectasia Gene and Breast Cancer

Ataxia-telangiectasia (AT) is an autosomal recessive syndrome of progressive cerebellar ataxia, immune deficiency and oculo-cutaneous telangiectasia associated with a high incidence of lymphoid malignancy. Heterozygotes for the AT gene are five times more likely to develop breast cancer than are noncarriers of the gene and appear to be particularly sensitive to the effects of ionizing radiation.(35) Approximately 1.5% of the general population are AT heterozygotes,(36) with the gene possibly accounting for up to 8% of all breast cancers. These data suggest that radiation-induced breast cancer may occur preferentially in women with Hodgkin's disease who also happen to be AT heterozygotes. A single gene responsible for AT has recently been identified and produces a product similar to phosphatidylinositol-3' kinase.(37) Study of this gene will allow a clearer understanding of mechanisms of radiation-induced carcinogenesis, and in the future may allow the prospective identification of patients at particularly high risk.

2.3.3 BRCA1 and Breast Cancer

BRCA1 is a gene located on chromosome 17q21 which is mutated in a subset of women with hereditary breast and ovarian cancer. Women carrying germline BRCA1 mutations (estimated to be 1 in 200 to 1 in 400 people in the United States) have an 85% lifetime risk of breast cancer often occurring before the age of 50.(38) In a recent study, four unrelated families have been shown to share a mutation designated 5382insC in exon 20 of the BRCA1 gene, with one family reporting both breast cancer and HD.(39) This specific BRCA1 mutation may be important in the etiology of both diseases.

2.4 Significance of the Planned Research

With current therapies, 90% of pediatric HD patients are cured of their cancer.(1) Current data suggest that approximately 35% of the female HD survivors are going to develop secondary breast cancer by the time they are 40 years of age. It is therefore very important to identify risk factors for the development of secondary breast cancer, those related both to HD treatment (age at radiation exposure and dose of radiation) and to genetic susceptibility (p 53, BRCA1, ATM). This information is needed in order to consider instituting measures for early detection (in the form of active screening, specifically mammographies), chemoprevention and modification of therapy for HD.

3.0 PRELIMINARY STUDIES

3.1 Increased Risk of Breast Cancer following Childhood Hodgkin's Disease: We followed a cohort of 1380 children with HD (diagnosed at one of the participating LESG institutions) for an average of 10.7 years to determine the incidence of second malignant neoplasms and associated risk factors. Eighty-eight second malignancies occurred in this cohort (Standardized Incidence Ratio (SIR), 18.1, 95% confidence interval (CI), 14.3-22.3). Breast cancer was the most common solid tumor (n=17; median age at diagnosis of breast cancer: 31.5 years; median latency from diagnosis of HD: 19.3 years). (Appendix Table 1) **HD survivors were at a 75-fold increased risk of developing breast cancer when compared to the general population (SIR, 75.3, 95% CI, 44.9-118.4). The actuarial estimated cumulative probability of developing breast cancer was 35±9% at 40 years of age for the cohort of female HD survivors**. Multivariate analysis revealed older age at diagnosis of HD (RR=1.7, p=0.03) and dose of radiation to be independently associated with increased risk (RR=5.9,p=0.03 for radiation dose between 2000 and 4000 cGy; RR=23.7, p=0.009 for radiation dose exceeding 4000 cGy).

4.0 RESEARCH DESIGN AND METHODS

4.1 Patient Eligibility: i) Diagnosis of HD at one of the LESG institutions between 1955 and 1986; ii) Age less than 16 years at diagnosis of HD; iii) Female gender.

4.1.1 Control Selection: Controls for Specific Aims 1 and 2 will be identified from the remaining population of female Hodgkin's disease survivors using the following criteria for matching:

    i) age at diagnosis of Hodgkin's disease ($\pm$ 1 year)   iii) radiation to mantle area

    ii) length of follow-up following Hodgkin's disease ($\pm$ 1 yr)   iv) primary institution

All study participants will be required to sign a written informed consent form, approved by the Institutional Review Board of their institution

4.2 Methods - Specific Aim 1

4.2.1 Family Histories

Pedigrees will be constructed including all first and second degree relatives of the proband, by using the detailed family history approach.(54). A chronological listing of all first and second degree relatives will be obtained and information obtained on demographic factors, vital status of the person (if deceased, the cause of death and age; if alive, inquiry will be made into his or her medical history). If the person has a history of breast and or ovarian cancer, information will be obtained about the site and type of cancer, age at diagnosis and the hospital where the diagnosis was made. All positive reports of cancer will then be validated from available hospital or medical records. This information will also be used to determine the incidence of cancer in the families (data analysis section). A summary family history (FH) score for each family would be obtained (data analysis section). In addition, the participants will be asked questions concering age at menarche and menopause, reproductive history, smoking habits, the regular use of any medicines during the previous 6 months, endocrine disorders and years of education. They would also be asked to give their most current height and weight for th purpose of calculating body mass index.

4.3 Methods - Specific Aim 2.

Blood samples from the surviving cases and from all the controls will be obtained by the respective institutions and shipped to the University of Minnesota. Samples will be coded by number prior to analysis and investigators will be blinded to the case-control status. Study participants will be informed that results of the analysis will not be available on an individual basis.

4.3.1 Molecular Studies

1. p53 - Sample of tumor tissue (paraffin-embedded or frozen) will be obtained from the 17 patients already identified as having developed breast cancer after treatment for childhood HD. Tumor tissue will be studied for p53 mutation using immunochemistry and PCR-SSCP. Immunochemistry will be performed on paraffin embedded tissue using a purified mouse monoclonal antibody that recognizes wild-type and mutant p53 (clone DO-1, Oncogene Science). The presence of detectable p53 protein by immunochemistry has been correlated with the presence of mutation in the gene, and the distribution (nuclear and cytoplasmic) has been suggested to be important in the pathogenesis of breast cancer.(56) The paraffin embedded tissue will be dewaxed and then incubated with unlabeled primary monoclonal antibodies. Specifically bound antibody will then be visualized by incubation with a biotinylated secondary antibody followed by a preformed avidin-biotinylated horseradish peroxidase macromolecular complex and substrate. Samples will be examined by light microscopy and the presence of p53 staining and its distribution recorded and compared with positive and negative controls provided by the manufacturer. PCR-SSCP will be used to identify sites of mutation in the p53

8

gene, which will then be characterized by direct DNA sequencing. DNA will be extracted from paraffin-embedded tissue using standard techniques. Briefly, 10 micron slices will be prepared from paraffin blocks in a sterile manner. Samples will then be chopped into small fragments with a fresh sterile scalpel blade for each sample, deparaffinized with xylene, rehydrated in TEN buffer (10 microm Tris, HCl pH 7.5, 2 mM EDTA and 100 mM NaCl) and digested overnight with proteinase K. Samples will then be extracted with phenol-chloroform, ethanol precipated, washed with 70% ethanol, dried and resuspended in TE buffer for amplification. DNA will be similarly extracted from frozen tissue by homogenization followed by proteinase K digestion, phenol extraction and ethanol precipitation. PCR amplification of exons 4 to 10 of the p53 gene will be performed using six different sets of primers to generate fragments of a suitable size for SSCP, as described by Murakami et al.(57) Briefly, the 5' ends of primers will be labeled by the polynucleotide kinase reaction with $[^{32}P]ATP$. The DNA samples (100 ng) will be subjected to PCR using each primer pair. Five microliters of the PCR product will then be mixed with formamide dye (95% formamide, 20mm EDTA, 0,05% xylene cyanol and 0.05% bromophenol blue), heated to 80 degrees Centigrade and applied to a 0.5XMDE (mutation detection enhancement, AT Biochem) gel. Samples will then be dried on filter paper and exposed to x-ray film for 12 hours. DNA fragments showing a mobility shift by PCR-SSCP analysis will be subjected to direct sequencing using dideoxy chain termination as previously described to characterize the mutation and distinguish polymorphisms.

    2. AT*M* - A cDNA clone representing part of the coding sequence of the gene mutated in ataxia telangiectasia has recently been isolated and the sequence deposited in Genbank.(37) We will screen study participants for mutations in this cDNA by extraction of RNA and RT-PCR followed by SSCP, as previously described.(37) Total RNA will be extracted from peripheral blood leukocytes or frozen tumor tissue with the Tri-reagent system (Molecular Research Center, Cincinnati, OH) and reverse transcribed with Superscript II reverse transcriptase (Gibco-BRL, Gaithersburg, MD) and an oligo-(dT) primer. The reaction products will serve as template for gene-specific primers which will be devised from the known sequence of AT*M* and used for PCR amplification and SSCP analysis. Fragments with abnormal migration identified by SSCP will be sequenced as described above. It is estimated that approximately 20 primer pairs will be needed to cover the 5.9 kb of known sequence. As genomic sequence of the ATM becomes available, genomic primers will be devised and utilized to look for somatic mutations of the ATM gene in paraffin-embedded tumor tissue.

    3. BRCA1 - Peripheral leukocytes and tumor tissue from all study participants will be screened for mutations in exon 20 of BRCA1. DNA will be extracted, amplified using specific primers as described by Simard et al,(58) and screened for mutation using SSCP as described above. Fragments with abnormal mobility will be directly sequenced to characterize the mutation. In patients with a high Family History Score (methods for Specific Aim 1), the entire BRCA1 coding sequence will be screened for germline and somatic mutation by PCR-SSCP as described by Simard et al.(58)

4.3.3 Controls Subjects

    Samples of peripheral blood will also be obtained from control HD patients who have not developed breast cancer. These samples will also be studied using PCR-SSCP for germline mutations in p53 and exon 20 of BRCA1, and by RT-PCR and SSCP for mutations in AT*M*.

4.4 Methods - Specific Aim 3.

    All patients who were alive at the time of the last update will be identified, and a questionnaire will be sent to the physician in the respective institutions. Information to be gathered includes: 1) date of last contact, 2) vital status of the patients at last contact, 3) development of neoplasm since the last contact, 4) recurrence of HD. patients newly diagnosed with breast cancer will be incorporated into the study, and consent obtained for construction of pedigrees and procuring blood and tissue samples for identifying somatic and/or germline mutations in the candidate genes.

5.0 DATA ANALYSIS

5.1 Specific aim 1: The expected number of affected family members based on demographic information (age, sex, race, and possibly birth cohort) will be calculated for both the cases (HD/breast cancer) and the controls (HD). Estimates of cumulative incidence rates derived from appropriate population surveys (SEER registry, and registries from other countries representing the case-control families) will be multiplied by the total person-years at risk for the family to calculate the expected number of cases for a family. Person-years at risk are accumulated from birth until age at interview or age at death for persons without cancer, or age at diagnosis for persons with breast cancer. Gender, race, age and time-specific incidence rates will be used to compute the expected number of cases. This expected number (E_i) for the ith family is then compared to the observed number (Oi) to give a summary family history (FH) score for this family as

$FH_i = O_i - E_i/(E_i)^{1/2}$ (where Oi =_O_{ij} and Ei = _E_{ij} for all j members of the ith family).(55) Family history scores directly quantitate the risk of disease in a family, but they can also be categorized into groups of essentially negative family history (FH<0.5), mild positive family history (1.0<FH<2.0), and very strong family history (FH>2.0).(55) Analysis of variance and t-tests will be employed to compare scores in the two groups of patients. Analyses will be performed with the Epilog software.(59)

5.2 Specific Aim 2: Conditional logistic regression will form the basis of most statistical analysis for cases and their matched controls. Three groups of variables will be defined: predominantly hereditary factors (family history, body height), reproductive factors (age at menarche, age at menopause, when applicable, reproductive history) and body measurements. Within these groups, a forward stepwise analysis based on comparison of p-values will be performed to identify risk factors. Relative Risk based on odds ratio will be tested for trend and linearity. In testing a particular variable only those study participants will be excluded, who have missing values for that variable or for those already included in the model.

6.0 PROJECTS COMPLETED AS OF JUNE 1997

As of June 1997, I have completed the construction of pedigrees for families of patients with secondary breast cancer. Pedigrees were constructed including all first and second degree relatives of the proband, by using the detailed family history approach. A chronological listing of all first and second degree relatives were obtained and information obtained on demographic factors, vital status of the person (if deceased, the cause of death and age; if alive, inquiry was made into his or her medical history). If the person had a history of breast and or ovarian cancer, information was obtained about the site and type of cancer, age at diagnosis and the hospital where the diagnosis was made. The expected number of affected family members based on demographic information (age, sex, race, and possibly birth cohort) was calculated for the cases (HD/breast cancer). Estimates of cumulative incidence rates derived from appropriate population surveys (SEER registry) were multiplied by the total person-years at risk for the family to calculate the expected number of cases for a family. Person-years at risk were accumulated from birth until age at interview or age at death for persons without cancer, or age at diagnosis for persons with cancer. This information was used to determine the incidence of cancer in the families (data analysis section). Analysis of the data collected form these families reveals no excess risk compared to the general population (manuscript enclosed). I have obtained breast cancer tissue from most of the cases, and am in the process of looking for somatic p53 mutations in the breast tissue. Blood is being obtained from the surviving patients with secondary breast cancer, in order to identify mutations in the ATM gene. In addition, I am in the process of completing a manuscript on recommendations for screening female survivors of Hodgkin's Disease for early detection of secondary breast cancer.

Conclusion

In an expanded assessment of the 13 cases with breast cancer developing at a very young age following treatment for Hodgkin's disease, we failed to demonstrate any evidence of familial aggregation of cancer (breast or otherwise) among family members. However, the influences of other well-established risk factors for the development of breast cancer, and biomarkers of genetic susceptibility (mutations in candidate genes), need to be explored in future studies, in order to identify high risk populations.

Family History of Cancer in Patients with Breast Cancer diagnosed following Treatment of Hodgkin's Disease in Childhood

Smita Bhatia, MD, MPH; Anna T Meadows, MD; Leslie L Robison, Ph.D.

In a recent study of the Late Effects Study Group (LESG),[1] we had observed an increased risk of breast cancer among female survivors of Hodgkin's disease diagnosed in childhood (standardized incidence ratio 75.3), with the estimated actuarial incidence approaching 35 percent by 40 years of age. Age at time of radiation (10 to 16 years: relative risk 1.7) and radiation dose (relative risk 5.9) were associated with significantly increased risk. Since then, there have been other reports of breast cancer developing after treatment of Hodgkin's disease in childhood, with the relative risks ranging from 17 to 61 times that of the general population. [2,3]

Prior studies[1,4] have shown that pubertal breast tissue is especially sensitive to the carcinogenic effects of ionizing radiation. However, the influence of well-established risk factors for breast cancer (e.g. a family history, etc.) on the development of radiation-associated tumors have not been explored thus far.

We conducted a study to evaluate the role of genetic predisposition (as measured by family history of cancer) in the development of breast cancer among the LESG cohort of survivors of Hodgkin's disease in childhood. Of 17 women with secondary breast cancer identified in this cohort,[1] 13 probands (76%) or their surviving next of kin were available for construction of pedigrees. The median age at diagnosis of Hodgkin's disease for these patients was 13 years (range, 7 to 15 years), and that for breast cancer was 34 years (range, 24 to 40 years). Nineteen family members were reported to have experienced cancer among the 180 first- and second-degree relatives (total follow-up of 9,351 person-years). Observed and expected cases (using cancer incidence rates from the SEER Registry[5]), standardized incidence ratios and 95% confidence intervals were calculated.

Overall, there was a significantly decreased risk of cancer among the family members (SIR=0.6, 95% CI, 0.4-0.9) (Table 1). Breast cancer was reported in three family members (median age at diagnosis, 59.5 years; range, 46 to 70 years). There was no excess of breast cancer overall, or in any of the subgroup of relatives examined.

In an expanded assessment of the 13 cases with breast cancer developing at a very young age following treatment for Hodgkin's disease, we failed to demonstrate any evidence of familial aggregation of cancer (breast or otherwise) among family members. However, the influences of other well-established risk factors for the development of breast cancer, and biomarkers of genetic susceptibility (mutations in candidate genes), need to be explored in future studies, in order to identify high risk populations.

TABLE 1. RISK OF CANCER IN RELATIVES OF PATIENTS WITH SECONDARY BREAST CANCER* ACCORDING TO AGE OF PROBAND AND RELATIONSHIP TO PROBAND.

HISTORY OF CANCER IN FAMILY MEMBERS	OBSERVED	EXPECTED	SIR† (95% CI)‡
All Relatives	19	30.9	0.6 (0.4-0.9)
Relatives of probands ≤ 13 yrs at Dx of HD	10	12.3	0.8 (0.4-1.4)
Relatives of probands > 13 yrs at Dx of HD	9	18.6	0.5 (0.2-0.9)
Relatives of probands ≤ 34 yrs at Dx of breast ca	13	12.7	1.0 (0.5-1.7)
Relatives of probands > 34 yrs at Dx of breast ca	6	18.2	0.3 (0.1-0.6)
First-degree Relatives	3	5.8	0.5 (0.1-1.3)
Maternal Relatives	13	13.2	1.0 (0.5-1.6)
Paternal Relatives	6	17.1	0.4 (0.1-0.7)

* Patients belong to the LESG cohort[1]
† SIR denotes standardized incidence ratios
‡ CI denotes confidence interval

References

1. Bhatia S, Robison LL, Oberlin O, et al. Breast cancer and other second neoplasms after childhood Hodgkin's disease. N Engl J Med 1996;334:745-51.
2. Sankila R, Garwicz S, Olsen JH, et al. Risk of subsequent malignant neoplasms among 1,641 Hodgkin's disease patients diagnosed in childhood and adolescence: a population-based cohort study in the five Nordic countries. J Clin Oncol 1996;14:1442-6.
3. Travis LB, Curtis RE, Boice JD. Late effects of treatment for childhood Hodgkin's disease. N Engl J Med 1996;335:352-3 (letter).
4. United Nations Scientific Committee on the Effects of Atomic Radiation. Sources and effects of ionizing radiation: UNSCEAR 1994 report to the General Assembly, with scientific annexes. New York: United Nations, 1994. (E.94.IXII).
5. Miller BA, Ries LAG, Hankey BF, et al (eds): SEER Cancer Statistics Review: 1973-1990, National Cancer Institute. NIH Pub. No. 93-2789,1993.

7.0 CONCLUSION

In an expanded assessment of the 13 cases with breast cancer developing at a very young age following treatment for Hodgkin's disease, we failed to demonstrate any evidence of familial aggregation of cancer (breast or otherwise) among family members. However, the influences of other well-established risk factors for the development of breast cancer, and biomarkers of genetic susceptibility (mutations in candidate genes), need to be explored in future studies, in order to identify high risk populations.

8.0 LITERATURE CITED

1. Miller BA, Ries LAG, Hankey BF, Kosary CL, Harras A, Devesa SS, Edwards BK (eds). SEER Cancer Statistics Review: 1973-1990. National Cancer Institute. NIH Pub. No. 93-2789, 1993.
2. DeVita VT, Serpick AA, Carbone PP: Combination chemotherapy in the treatment of advanced Hodgkin's disease. Ann Intern Med 1970;73:881-95.
3. Horning SJ, Hoppe RT, Kaplan HS, Rosenberg SA. Female reproductive potential after treatment for Hodgkin's disease. N Engl J Med 1981;304:1377-82.
4. Sherins RJ, DeVita Jr. Effect of drug treatment for lymphoma on male reproductive capacity: studies of men in remission after therapy. Ann Intern Med 1973:;79:216-20.
5. Rosenberg SA, Kaplan HS. The evolution and summary results of the Stanford randomized clinical trials of the management of Hodgkin's disease:1962-1984. Int J Radiat Oncol Biol Phys 1985;11:5-22.
6. Arseneau JC, sponzo RW, Levin DL, et al. Nonlymphomatous malignant tumors complicating Hodgkin's disease: Possible association with intensive therapy. N Engl J Med 1972;287:1119-22.
7. Canellos GP, DeVita VT, Arseneau JC, et al. Second mailgnancies complicating Hodgkin's disease in remission. Lancet 1975;1:947-49.
8. Coleman CN, Kaplan HS, Cox R, et al. Leukemias, non-Hodgkin's lymphomas and solid tumors in patients treated for Hodgkin's disease. Cancer surveys 1982;1:733-34.
9. Coltman CA, Dixon DO. Second malignancies complicating Hodgkin's disease: A Southwest Oncology Group 10-year follow-up. Cancer Treat Rep 1982;66:1023-33.
10. Glicksman AS, Pajak TF, Gottlieb JA, et al. Second malignant neoplasms in patients successfully treated for Hodgkin's disease: a cancer and leukemia group B study. Cancer Treat Rep 1982;66:1035-44.
11. Boivin JF, Hutchinson GB, Lyden M, et al. Second primary cancers following treatment of Hodgkin's disease. JNCI 1984;72:233-41.
12. Koletsky AJ, Bertino JR, Farber LR, Prosnitz LR, Kapp DS, Fischer D, Portlock CS. Second neoplasms in patients with Hodgkin's disease following combined modality therapy: The Yale experience. J Clin Oncol 1986;4:311-17.
13. Valagussa P, Santoro A, Fossati-Bellani F, Bonadonna G. Second acute leukemia and other malignancies following treatment for Hodgkin's disease. J Clin Oncol 1086;4:830-37.
14. Blaney DW, Longo DL, Young RC, Greene MH, Hubbard SM, Postal MG, Duffey PL, DeVita VT. Decreasing risk of leukemia with prolonged follow-up after chemotherapy and radiotherapy for Hodgkin's disease. N Engl J Med 1987;316:710-14.
15. Pederson-Bjergaard J, Larsen SO, Struck J, Hansen HH, Specht L, Hansen MM, Nissen NI. Risk of therapy-related leukemia and preleukemia after Hodgkin's disease. Lancet 1087;2:83-88.
16. Tucker MA, Meadows AT, Boice JD, et al. Leukemia after therapy with alkylating agents for childhood cancer. JNCI 1987;78:459-64.
17. Meadows AT, Obringer AC, Marrero O, et al. Second malignant neoplasms following childhood Hodgkin's disease: Treatment and splenectomy as risk factors. Med Pediatr Oncol 1989;17:477-84.
18. Beaty O, Hudson MM, Greenwals C, et al. Subsequent malignancies in children and adolescents after treatment for Hodgkin's disease. J Clin Oncol 1995;13:603-9.
19. Boivin JF, Hutchinson GB, Zauber AG, et al. Incidence of second cancers in patients treated for Hodgkin's disease. JNCI 1995;87:732-41.
20. MacKenzie I. Breast cancer following multiple fluoroscopies. Br J Cancer 1965:19:1-8.
21. Miller AB, Howe GR, Sherman GJ, et al. Mortality from breast cancer after irradiation during fluoroscopic examinations in patients being treated for tuberculosis. N Engl J Med 1989;321:1285-89.
22. Shore RE, Hildreth N, Woodard E, et al. Breast cancer among women given x-ray therapy for acute post-partum mastitis. JNCI 1986;77:689-96.

23. Baral E, Larsson LE, Mattson B. Breast cancer after irradiation of the breast. Cancer 1977;40:2905-10.
24. Tokunaga M, Land CE, Yamamoto T, et al. Incidence of female breast cancer among atomic bomb survivors. Hiroshima and Nagasaki:1950-1980. Radiat Res 1987;112:243-72.
25. Boice JD, Land CE, Shore RE, et al. Risk of breast cancer following low dose radiation exposure. Radiology 1979;131:589-97.
26. Curtis RE, Boice JD Jr. Second cancer after radiotherapy for Hodgkin's disease. N Engl J Med1988;319:244-245.
27. Hancock SL, Tucker MA, Hoppe RT. Breast cancer after treatment of Hodgkin's disease. JNCI 1993;85:25-31.
28. Parker RG. Radiation-induced cancer as a factor in clinical decision making. Int J Radiat Oncol Biol Phys 1990;18:993-1000.
29. Elkind MM, Bedford JS, Benjamin SA, et al. Oncogenic mechanisms in radiation-induced cancer. Cancer Res 1991;51:2740
30. Claus EB, Risch N, Thompson WD. Age at onset as an indicator of familial risk of breast cancer. Am J Epidemiol 1990;131:961-72.
31. Claus EB, Risch N, Thompson WD. Autosomal dominant inheritance of early-onset breast cancer. Cancer 1994;73:643-51-47.
32. Malkin D, Li FP, Strong LC, et al. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science 1990;250:1233-38.
33. Schneider KA. Counselling about cancer: Strategies for genetic counselors. Boston, Mass: Dana Farber Cancer Institute;1994.
34. Lee JM, Abrahamson JLA, Kandel R, et al. Susceptibility to radiation-carcinogenesis and accumulation of chromosomal breakage in p53 deficient mice. Oncogene 1994;9:3731-36.
35. Swift M, Morrell D, Massey RB, et al. Incidence of cancer in 161 families affected by ataxia-telangiectasia. N Engl J Med 1991;325:1831-36.
36. Swift M, Morrell D, Cromartie E, et al. The incidence and gene frequency of ataxia-telangiectasia in the United States. Am J Hum Genet 1986;39:573-83.
37. Savitsky K, Bar-Shira A, Gilad S, et al. A single ataxia-telangiectasia gene with a product similar to PI-3 kinase. Science 1995;268:1749-53.
38. Easton DF, Bishop T and Ford D. Genetic linkage analysis in familial breast cancer and ovarian cancer: results from 214 families. Am J Hum Genet 1993;52:678-701.
39. Simard J, Tonin P, Durocher K, et al. Common origins of BRCA1 mutations in Canadian breast and ovarian cancer families. Nature Genetics 1994;8:392-98.
40. Moses AC, Pilstine SJ. Insulin-like growth factors. In: Boyton AC and Lefferts HL, eds. Control of Animal Cell Proliferation. New York, Academic Press, 1985, 1, 91-120.
41. Clemons DR, Van Wyk JJ. Factor controlling blood concentration of somatedin-C. Clin Endocrinol Metab 1984;13:113-43.
42. Vermeulen A. Nyctohemeral growth hormone profiles in young and aged men: correlation with somatedin-C levels. J Clin Endocrinol Metab 1987;64:884-8.
43. Macauley, VL. Insulin-like growth factors and cancer. Br J Cancer, 1992;65:311-20.
44. Yee D, Paik S, Leboric GS, Marcus RR, et al. Analysis of insulin-like growth factor I gene expression in malignancy: evidence for a paracrine role in human breast cancer. Mol Endocrinol 1989;3:509-17.
45. Brunning PF, Van Doorn J, Bonfrer JMG, Van Noord PAH, Korse CM, Linders TC, and Hart AAM. Insulin-like growth factor binding protein-3 is decreased in early stage operable pre-menopausal breast cancer. Int J Cancer 1995;62:266-70.
46. Torrisi R, Pensa F, Orengo MA, et al. The synthetic retinoid fenretinide lowers plasma insulin-like growth factor-I levels in breast cancer patients. Cancer Res 1993;53:4769-71.

47. Buchanan JB, Spratt JS, Heuser LS. Tumor growth, doubling times, and the inability of radiologists to diagnose certain cancers. Radiol Clin north Am. 1983;21:115-26.
48. von Fournier D, Weber E, Hoeffken W, et al. Growth rate of 147 mammary carcinomas. Cancer 1980;45:2198-2207.
49. Tubiana M, Koscielny S. The natural history of breast cancer: implications for a screening strategy. Int J Radiat Oncol Biol Phys.1990;19:1117-20.
50. Fletcher SW, Back W, Harris W, et al. Report of the International Workshop on Screening for Breast Cancer. J Natl Cancer Inst 1993;85:1644-56.
51. Kerliskowske K, grady D, Rubin SM, et al. Efficacy of screening mammography: a metanalysis. JAMA 1995;273:149-54.
52. Harris VJ, Jackson VP. Indications for breast imaging in women under age 35 years. Radiology 1989;172:445-8.
53. Vogel VG, Graves DS, Vernon SW, et al. Mammogarphic screening of women with increased risk of breast cancer. Cancer 1990;66:1613-20.
54. Epidemiological approaches to familial aggregation. In Fundamentals of Genetic Epidemiology. Eds Khoury MJ, Beaty TH, Cohen BH. New York Oxford, Oxford University Press. Pg 164-199, 1993.
55. Hunt SC, Hasstedt SJ, William RR. Testing for familial aggregation of a dichotomous trait. Genetic Epidemiology 1986;3:299-312.
56. Moll UM, Riou G and Levine AJ. Two distinct mechanisms alter p53 in breast cancer: mutation and nuclear exclusion. Proc Natl Acad Sci 1992;89:7262-66.
57. Mukarami Y, Hayashi K, Hirohashi S, et al. Aberrations of the tumor suppresser gene p53 and retinoblastoma genes in human hepatocellular carcinomas. Cancer Res 1991;51:5520-25.
58. Simard J, Tonin P, Durocher K, et al. Common origins of BRCA1 mutations in Canadian breast and ovarian cancer families. Nature Genetics 1994;8:392-98.
59. Buckley J: "Epilog. Epidemiology Statistics Package". Los Angeles: Epicenter Software,1986.

Table 1. Characteristics of the 17 patients with secondary breast cancer

LESGNO*	Age at HD**	Age at BC#	Years to BC	Status
252	6 yrs	34.5 yrs	28.5 yrs	Alive
256	12 yrs	16.3 yrs	4.3 yrs	Alive
257	14 yrs	22.3 yrs	8.2 yrs	Alive
448	15 yrs	28.7 yrs	13.7 yrs	Dead
454	11 yrs	32.1 yrs	21.1 yrs	Alive
596	13 yrs	15.4 yrs	2.4 yrs	Alive
606	15 yrs	37.3 yrs	22.3 yrs	Alive
629	14 yrs	39.0 yrs	25.0 yrs	Alive
642	15 yrs	37.1 yrs	22.1 yrs	Alive
674	14 yrs	27.1 yrs	13.1 yrs	Alive
701	12 yrs	38.4 yrs	26.4 yrs	Alive
756	12 yrs	36.2 yrs	24.2 yrs	Alive
914	15 yrs	25.0 yrs	10 yrs	Alive
2174	14 yrs	29.8 yrs	15.8 yrs	Dead
2175	14 yrs	42.0 yrs	28.0 yrs	Unknown
2176	12 yrs	36.3 yrs	24.3 yrs	Dead
2253	13 yrs	30.8 yrs	17.8 yrs	Unknown

*LESGNO denotes Late Effects Study Group Number   # BC denotes breast cancer

** Age at HD dnotes age at diagnosis of Hodgkin's disease

3.7 Late Effects Study Group

The Late Effects Study Group (LESG) consists of 15 institutions from the United States, Canada and Western Europe, and is involved in studying Long-Term Complications following childhood cancer. The following institutions are included in the LESG:

Dana-Farber Cancer Institute, Boston
Columbus Children's Hospital, Columbus
Children's Hospital of Philadelphia
Children's Memorial Hospital, Chicago
Roswell Park Memorial Institute, Buffalo
University of Minnesota, Minneapolis
Children's Hospital of Los Angeles, LA
Institut Gustave-Roussy, Villejuif, France

Children's Hospital Medical Center, Cincinnati
Children's NationalMedical Center, Washington DC
Children's Hospital of Pittsburgh
Hospital for Sick Children, Toronto
Emma KinderZiekenhuis, Amsterdam
Royal Manchester Children's Hospital, England
Istituto Nazionale Tumori, Milan, Italy

BREAST CANCER AND OTHER SECOND NEOPLASMS AFTER CHILDHOOD HODGKIN'S DISEASE

SMITA BHATIA, M.D., M.P.H., LESLIE L. ROBISON, PH.D., ODILE OBERLIN, M.D., MARK GREENBERG, M.B., CH.B., GRETA BUNIN, PH.D., FRANCA FOSSATI-BELLANI, M.D., AND ANNA T. MEADOWS, M.D.

Abstract *Background.* Patients who survive Hodgkin's disease are at increased risk for second neoplasms. As survival times increase, solid tumors are emerging as a serious long-term complication.

Methods. The Late Effects Study Group followed a cohort of 1380 children with Hodgkin's disease to determine the incidence of second neoplasms and the risk factors associated with them.

Results. In this cohort, there were 88 second neoplasms as compared with 4.4 expected in the general population (standardized incidence ratio, 18.1; 95 percent confidence interval, 14.3 to 22.3). The estimated actuarial incidence of any second neoplasm 15 years after the diagnosis of Hodgkin's disease was 7.0 percent (95 percent confidence interval, 5.2 to 8.8 percent); the incidence of solid tumors was 3.9 percent (95 percent confidence interval, 2.3 to 5.5 percent). Breast cancer was the most common solid tumor (standardized incidence ratio, 75.3; 95 percent confidence interval, 44.9 to 118.4),

with an estimated actuarial incidence in women that approached 35 percent (95 percent confidence interval, 17.4 to 52.6 percent) by 40 years of age. Older age (10 to 16 vs. <10 years) at the time of radiation treatment (relative risk, 1.9) and a higher dose (2000 to 4000 vs. <2000 cGy) of radiation (relative risk, 5.9) were associated with significantly increased risk of breast cancer. The estimated actuarial incidence of leukemia reached a plateau of 2.8 percent (95 percent confidence interval, 0.8 to 4.8 percent) 14 years after diagnosis. Treatment with alkylating agents, older age at the diagnosis of Hodgkin's disease, recurrence of Hodgkin's disease, and a late stage of disease at diagnosis were risk factors for leukemia.

Conclusions. The risk of solid tumors, especially breast cancer, is high among women who were treated with radiation for childhood Hodgkin's disease. Systematic screening for breast cancer could be important in the health care of such women. (N Engl J Med 1996;334:745-51.)
©1996, Massachusetts Medical Society.

LONG-TERM sequelae of the treatment of Hodgkin's disease are being encountered with increasing frequency because of the marked improvement in survival.[1-4] Second neoplasms, particularly acute myelogenous leukemia, are well-known late complications in patients who have been treated for Hodgkin's disease as adults.[5-15] An increased risk of second neoplasms in patients treated for Hodgkin's disease in childhood has also been reported by the Late Effects Study Group[16] and others.[17,18] In an earlier study, we estimated the cumulative probability of any second neoplasm to be 20 percent (4 percent for leukemia and 16 percent for solid tumors) 20 years after a diagnosis of Hodgkin's disease in childhood.[16] To investigate further the incidence of second neoplasms af-

ter the treatment of childhood Hodgkin's disease and to identify specific factors associated with the risk, we extended the median follow-up for the cohort of the Late Effects Study Group from 7 to 11.4 years and increased the size of the cohort from 979 to 1380.

METHODS

Fifteen institutions participated in this study (see the Appendix). The cohort consisted of children who were less than 16 years of age when their Hodgkin's disease was diagnosed and who received their primary treatment between 1955 and 1986 at a participating institution.

At each institution, a roster of all patients with Hodgkin's disease was prepared, and data were abstracted from the clinical records. Doses, fields, and equipment used in radiation therapy were noted, as were agents, doses, and durations of chemotherapy. For each patient, the date of last contact was obtained from the clinical records. For patients in whom second neoplasms developed, the date of diagnosis, the histologic characteristics and site of the tumor, and whether the tumor arose in the radiation-therapy field were recorded. If the patient died, the date and cause of death were also reported. Pathological findings were confirmed at the treating institution. The length of time at risk for second neoplasms was computed from the date of the diagnosis of Hodgkin's disease to the date of the diagnosis of the second neoplasm, the date of death, or the date of last contact, whichever came first.

For purposes of analysis, patients were classified in one of three mutually exclusive treatment groups. The first group received radia-

tion therapy alone, the second group received chemotherapy alone, and the third group received both radiation therapy and chemotherapy (the latter either as part of the primary treatment or as salvage therapy for recurrence).

Patients who were treated with alkylating agents were analyzed separately. The following drugs were included in that class: mechlorethamine hydrochloride, cyclophosphamide, chlorambucil, procarbazine, nitrosoureas, triethylenemelamine, thiotepa, and dacarbazine. A score for the doses of alkylating agents received by each patient[16] was calculated as follows: a single alkylating agent administered for at least six months was assigned a score of 1; two alkylating agents for six months, a score of 2; and so on. All such scores corresponding to the patient's treatment course were added together and rounded to the nearest integer.

To estimate the risk of second neoplasms, the number of person-years of observation was compiled for subgroups of the cohort defined by age and sex. Rates of incidence of cancer (obtained from the registry of the Surveillance, Epidemiology, and End Results Program of the National Institutes of Health[19]) were used to calculate the expected number of cases of cancer. Standardized incidence ratios were calculated as the ratios of observed to expected cases. The 95 percent confidence intervals were estimated by a method described by Vandenbroucke.[20] Cumulative probabilities of second neoplasms were calculated with actuarial methods.[21] Cox regression techniques were used to calculate estimates of relative risk. Variables included in the regression model were sex, age at the diagnosis of Hodgkin's disease, clinical stage of the disease, treatment group, whether splenectomy had been performed, the alkylating-agent score, and the dose of radiation. Recurrence was included as a time-dependent covariate in the regression model. Age at the diagnosis of Hodgkin's disease was analyzed both as a categorical variable (less than 10 years or 10 to 16 years) and as a continuous variable. Clinical stages I and II and clinical stages III and IV were grouped because of the strong correlation between treatment and clinical presentation.

RESULTS

The median duration of follow-up was 11.4 years, and 80 percent of the cohort of 1380 eligible patients with Hodgkin's disease were alive at the time of last contact

Table 1. Characteristics of the Patients.

CHARACTERISTIC	TOTAL COHORT	PATIENTS WITH SECOND CANCER		
		SOLID TUMOR	LEUKEMIA	NON-HODGKIN'S LYMPHOMA
No. of patients	1380	56	26	6
Male sex — %	65	43	69	50
Stage of Hodgkin's disease — %				
I or II	65	76	31	67
III or IV	35	24	69	33
Age at diagnosis				
Median — yr	11	12	11	11
Range — yr	1–16	2–16	3–15	7–15
<10 yr — no. of patients (person-yr of follow-up)	504 (6025)	17	6	2
10–16 yr — no. of patients (person-yr of follow-up)	876 (9635)	39	20	4
Time to second cancer — yr				
Median	—	14	4	14
Range	—	0.8–28	0.8–14	0.8–18
Follow-up — yr				
Median	11.4	19	5	13
Range	0.1–37	4–36	2–15	1–23
Treatment — % of patients				
Radiation alone	23	30	0	17
Chemotherapy alone	8	2	19	17
Radiation and chemotherapy	69	68	81	66
Death — %	20	30	96	83

Table 2. Observed and Expected Rates of Second Cancers in the Entire Cohort, According to Type and Site.

TYPE OR SITE	OBSERVED CASES	EXPECTED CASES	STANDARDIZED INCIDENCE RATIO (95% CI)*
All cancers†	79	4.4	18.1 (14.3–22.3)
Leukemia	26	0.3	78.8 (56.6–123.2)
Acute myelogenous leukemia	24	0.1	321.3 (207.5–467.1)
Non-Hodgkin's lymphoma	6	0.3	20.9 (7.7–42.0)
Solid tumors‡	47	3.9	11.8 (8.7–15.4)
Breast§	17	0.2	75.3 (44.9–118.4)
Thyroid	10	0.3	32.7 (15.3–55.3)
Bone	4	0.2	24.6 (6.4–54.5)
Brain	4	0.4	10.5 (2.7–23.4)
Colorectal	3	0.1	38.9 (7.3–95.3)
Gastric	2	0.02	121.3 (11.4–145.2)

*CI denotes confidence interval.

†This category excludes the nine cases of nonmelanoma skin cancer.

‡This category excludes lymphatic and hematopoietic tumors. The sum of the solid tumors listed does not equal the total number given because only types for which the risk was significantly elevated are included.

§The cohort for this analysis included only women.

(Table 1). At the time data were abstracted, there had been documented contact with approximately 71 percent of the patients within the previous five years and with 54 percent of the patients within the previous two years. Treatment for Hodgkin's disease consisted of radiation and chemotherapy in 69 percent of the patients, radiation alone in 23 percent, and chemotherapy alone in 8 percent. Among the patients who received radiation therapy, orthovoltage techniques were used for treatment in only 2 percent.

Second neoplasms developed in 109 patients: 56 had solid cancers, 26 had leukemia, 6 had non-Hodgkin's lymphoma, and 21 had benign tumors. The benign tumors included 12 thyroid adenomas, 4 osteochondromas, 3 fibroadenomas of the breast, and 2 dysplastic nevi.

The numbers of observed and expected second cancers are shown in Table 2. There were significantly elevated relative risks for all cancers combined, for leukemia, for non-Hodgkin's lymphoma, and for breast, thyroid, bone, central nervous system, colorectal, and gastric cancers.

Figure 1 shows the actuarial risks of all second cancers, solid tumors, leukemia, and non-Hodgkin's lymphoma. The mean cumulative incidence of any second cancer was 7.0 percent (95 percent confidence interval, 5.2 to 8.8 percent) at 15 years. Most of this risk was due to solid tumors; the steep increase in the cumulative incidence of solid tumors began 12 years after the diagnosis of Hodgkin's disease, and the risk rose to 3.9 percent (95 percent confidence interval, 2.3 to 5.5 percent) at 15 years. In contrast, the risk of leukemia reached a plateau at 2.8 percent (95 percent confidence interval, 0.8 to 4.8 percent), and the risk of non-Hodgkin's lymphoma plateaued at 1.1 percent (95 percent confidence interval, 0 to 3.1 percent).

We also estimated the standardized incidence ratio for cancer according to the period of observation (i.e., the interval from first treatment to the diagnosis of a

second cancer) (Table 3). The standardized incidence ratio was highest during the first five years of follow-up and gradually declined thereafter. This phenomenon is consistent with the increase in the expected incidence of cancer with increasing age. For leukemia, the excess risk appeared within the first 5 years of treatment and declined over the next 10 years of follow-up. No cases of leukemia were observed beyond 15 years after the diagnosis of Hodgkin's disease.

Leukemia

Leukemia developed in 26 patients. Twenty-four of them had acute myeloid leukemia, one had acute lymphoblastic leukemia, and one had chronic myeloid leukemia. There were no cases of leukemia in the group treated only with radiotherapy. The cumulative risks of leukemia (at 15 years) were higher in the group of patients who received chemotherapy alone (7.9 percent; 95 percent confidence interval, 1.0 to 14.8 percent) than among the patients who were treated with both radiation and chemotherapy (3.4 percent; 95 percent confidence interval, 1.8 to 4.9 percent) (Table 4).

The risk of leukemia rose with an increase in the alkylating-agent score (relative risk of leukemia per unit increase in the score, 1.5; 95 percent confidence interval, 1.2 to 1.8). Among the 340 patients who received a combination of mechlorethamine, vincristine, procarbazine, and prednisone, the cumulative probability of leukemia 15 years after the diagnosis of Hodgkin's disease was 2.9 percent (95 percent confidence interval, 0.7 to 5.1 percent), as compared with 0.9 percent (95 percent confidence interval, 0 to 9.5 percent) among the 103 patients who received a combination of doxorubicin, bleomycin, vinblastine, and dacarbazine. Univariate analysis revealed that patients were at increased risk for leukemia if they had had one or more recurrences of Hodgkin's disease (relative risk, 2.3; 95 percent confidence interval, 1.2 to 5.2), a later stage (III or IV) at diagnosis (relative risk, 4.2; 1.7 to 10.3), or an older age (10 to 16) at the diagnosis of Hodgkin's disease (relative risk, 3.6; 1.1 to 12.2). The risk of leukemia was not significantly increased in the subjects who had undergone splenectomy (relative risk, 1.4; 95 percent confidence interval, 0.6 to 3.4). Of the 572 patients who underwent splenectomy, 13 had leukemia, as compared with 9 of the 637 patients who did not undergo splenectomy.

Multivariate analysis revealed that a late stage of Hodgkin's disease at diagnosis and recurrent disease independently predicted the risk of secondary leukemia. However, patients presenting with late-stage disease had a significantly higher mean (±SE) alkylating-agent score than those presenting with early-stage disease (2.4±0.06 vs. 1.2±0.04, P<0.001). Similarly, patients with recurrent Hodgkin's disease had received significantly higher cumulative doses of alkylating agents than patients with no recurrence (mean score, 2.5±0.08 vs. 1.2±0.03; P<0.001). In addition, patients who presented with late-stage disease and had also had a recurrence had significantly higher alkylating-agent scores than patients who present-

ed with early-stage disease and had no subsequent recurrence (mean score, 3.4±0.1 vs. 0.9±0.04; P<0.001).

Of the 26 patients with leukemia, 25 died; the median survival was 2.5 months after the diagnosis of leukemia. Twenty-three patients died of secondary leukemia, one in an accident, and one of progressive Hodgkin's disease.

Lymphomas

Non-Hodgkin's lymphoma developed in six patients. The alkylating-agent score was the only significant independent risk factor for non-Hodgkin's lymphoma (relative risk, 1.7; 95 percent confidence interval, 1.2 to 2.6). Five patients with non-Hodgkin's lymphoma died; the median survival was 2.5 months. Four died of the non-Hodgkin's lymphoma, and one of progressive Hodgkin's disease.

Solid Cancers

Solid cancers developed in 56 patients. Breast cancer was the most common solid tumor, occurring in 17 patients. Ten patients had thyroid cancer, nine had basal-cell carcinomas, four had bone tumors, four had brain tumors, and three had colorectal carcinomas. Gastric carcinomas, tumors of the female genitourinary tract, parotid-gland tumors, soft-tissue sarcomas, and neuroblastoma occurred in one or two patients each. Risk factors were analyzed both with and without the inclusion of basal-cell carcinomas. There was no difference between the results of the two analyses, and so those of the latter are reported.

Sixty-six percent of the solid cancers developed in the group of patients who had received both radiation and chemotherapy (Table 4). The estimated cumulative probability of a solid tumor 20 years after the diagnosis of Hodgkin's disease was significantly higher among women (12.6 percent; 95 percent confidence interval, 6.8 to 18.4 percent) than men (3.9 percent; 1.5 to 6.3 percent). When the 17 women with breast cancer were excluded, the cumulative probability of solid tumors among the women in the group (8.8 percent; 95 percent
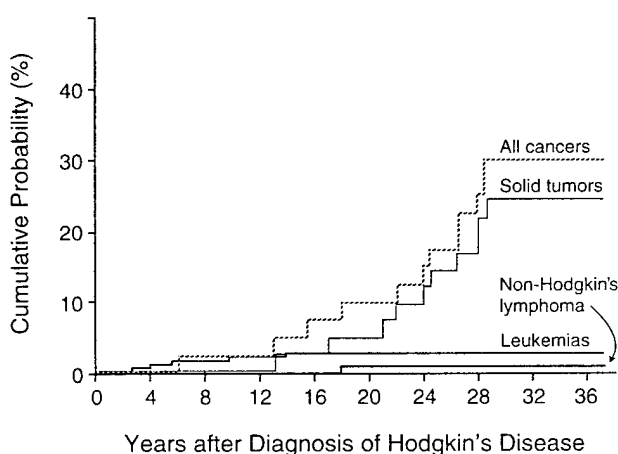

Figure 1. Cumulative Probability of Second Cancers in 1380 Patients with Hodgkin's Disease in Childhood.

Table 3. Standardized Risk Ratios for Second Cancers, According to the Length of the Follow-up Interval.

TYPE OF CANCER*	LENGTH OF FOLLOW-UP				
	0–5 YR	6–10 YR	11–15 YR	16–20 YR	>20 YR
All cancers					
Observed	29	15	17	8	10
Observed:expected (95% CI)	28.0 (18.8–39.2)	17.9 (10–28.5)	15.3 (8.9–23.5)	6.7 (2.9–12.2)	35.9 (17.1–61.7)
Leukemia					
Observed	18	6	2	0	0
Observed:expected (95% CI)	99.6 (58.9–150.9)	83.3 (29.9–163.3)	37.3 (3.5–106.9)	0	0
Non-Hodgkin's lymphoma					
Observed	2	2	1	1	0
Observed:expected (95% CI)	24.6 (2.3–70.6)	33.1 (3.1–94.7)	13.3 (0–52.3)	12.6 (0–49.5)	0
Solid tumors					
All					
Observed	9	7	14	7	10
Observed:expected (95% CI)	11.6 (5.2–20.5)	10 (3.9–18.7)	14.3 (7.8–22.2)	6.5 (2.6–12.2)	39.7 (18.9–68.1)
Breast					
Observed	2	2	4	1	8
Observed:expected (95% CI)	4950.5 (466.7–14,188.8)	231.8 (21.8–664.3)	76.2 (19.8–169.2)	7.5 (0–29.6)	141.5 (60.4–256.5)
Thyroid					
Observed	1	3	4	2	0
Observed:expected (95% CI)	18.7 (0–73.2)	41.1 (7.7–100.7)	40.9 (10.6–90.8)	21.5 (2.0–61.7)	0

*Observed denotes the number of cases observed, observed:expected the ratio of observed to expected cases, and CI confidence interval.

confidence interval, 3.4 to 14.2 percent) approached that among the men (3.9 percent; 1.5 to 6.3 percent). Multivariate analysis revealed that female sex was associated with an increased risk of solid tumors (relative risk, 2.9; 95 percent confidence interval, 1.5 to 5.4). Older patients (those 10 to 16 years of age at the diagnosis of Hodgkin's disease) also appeared to be at increased risk for solid tumors (relative risk as compared with those <10 years at diagnosis, 1.8; 95 percent confidence interval, 0.96 to 4.0). Exclusion of the nine patients with basal-cell carcinoma made this association nonsignificant (relative risk, 1.6; 95 percent confidence interval, 0.8 to 3.1).

Seventeen of the 56 patients with solid tumors died. The median survival was 12.5 months after the diagnosis of the second neoplasm; 10 deaths were due to the second neoplasm and 7 to accidents.

Breast Cancer

Of the 17 women in whom breast cancer developed, 7 had received radiation therapy alone and 10 had received radiation and chemotherapy. Of the 17 cancers, 16 appeared within or at the margin of the radiation field. In one patient, the tumor (a multifocal infiltrating ductal carcinoma) occurred outside the radiation field (the patient had received radiation to the neck). Five patients had bilateral breast tumors. The majority of the tumors were infiltrating ductal or lobular carcinomas. The median age at the time of diagnosis of breast cancer was 31.5 years (range, 16 to 42). Three patients died of their breast cancer (median survival, 3 years), eight were alive with disease at this writing (median length of follow-up after diagnosis, 10 months), four were alive without dis-

ease (median length of follow-up, 4.5 years), and the status of two was unknown.

The women in our cohort of survivors of Hodgkin's disease had a risk of breast cancer that was 75 times the risk in the general population (Table 2). The risk of breast cancer was elevated throughout the follow-up period, and the interval from the diagnosis of Hodgkin's disease to the diagnosis of breast cancer was less than five years in two cases (Table 3). Figure 2 shows the estimated cumulative probability of breast cancer as a function of the age of the cohort of female survivors of Hodgkin's disease. The estimated actuarial cumulative probability of breast cancer was 35 percent (95 percent confidence interval, 17.4 to 52.6 percent) at 40 years of age.

Univariate analysis revealed that patients who were 10 to 16 years of age when Hodgkin's disease was diagnosed and treated were at increased risk for breast cancer as compared with those who were younger than 10 at diagnosis (relative risk, 6.7; 95 percent confidence interval, 1.2 to 28.6). In addition, patients who underwent splenectomy appeared to be at increased risk for breast cancer (relative risk, 2.6; 95 percent confidence interval, 0.96 to 5.0). Patients with breast cancer received a higher dose of radiation to the mantle region (median, 4000 cGy; range, 0 to 4750) than those in whom breast cancer did not develop (median, 2000 cGy; range, 0 to 5200). Seventy-six percent of the patients who had breast cancer had received at least 2000 cGy of radiation to the mantle region, as compared with 48 percent of the patients who did not have breast cancer.

Multivariate analysis revealed that an age of more than 10 years at the time of diagnosis of Hodgkin's dis-

Table 4. Risks of Second Cancers According to the Type
of Treatment for Hodgkin's Disease.*

Type of Cancer and Treatment	Observed Cases	Observed:Expected Cases (95% CI)	Cumulative Probability at 15 yr (95% CI)
			%
Leukemia			
Radiation	0	0	0
Chemotherapy	5	1091 (344–2256)	7.9 (1.0–14.8)
Radiation and chemotherapy	21	439 (270–645)	3.4 (1.8–4.9)
Non-Hodgkin's lymphoma			
Radiation	1	11 (0.01–44)	0.4 (0–1.2)
Chemotherapy	1	60 (0.02–235)	0.0
Radiation and chemotherapy	4	23 (6–50)	0.9 (0–1.9)
Solid tumors			
Radiation	15	11 (6–17)	3.3 (2.9–3.7)
Chemotherapy	1	5 (0.01–18)	2.9 (2.3–3.5)
Radiation and chemotherapy	31	13 (9–18)	4.6 (4.4–4.8)

*CI denotes confidence interval.

ease was independently associated with increased risk (relative risk, 1.9; 95 percent confidence interval, 1.1 to 3.2), as was a higher dose of radiation (as compared with a radiation dose of <2000 cGy, the relative risk for a dose between 2000 and 4000 cGy was 5.9 [1.2 to 30.3], and the relative risk for a dose exceeding 4000 cGy was 23.7 [3.7 to 152.3]).

DISCUSSION

Among the 1380 patients who were treated for childhood Hodgkin's disease between 1955 and 1986 at 15 institutions, we found the estimated cumulative risk of a second cancer to be 7.0 percent 15 years after the initial diagnosis. This report provides evidence that the risk of a second neoplasm is increased about 18 times in long-term survivors of childhood Hodgkin's disease. The risk was highest in patients who were older when they had Hodgkin's disease, with 74 percent of the cancers occurring in those who received diagnoses between 10 and 16 years of age. This finding is similar to that reported by Beaty et al.[17]

Breast cancer was the most common solid tumor in this group of patients. The women in our cohort had a risk of breast cancer 75 times greater than that in the general population. Moreover, the estimated cumulative probability of breast cancer among women in our cohort who survived childhood Hodgkin's disease approached 35 percent at 40 years of age. For our multinational investigation, we used the rates of the U.S. Surveillance, Epidemiology, and End Results Program for the incidence of breast cancer in the general population[19] because the age-standardized rates for France (66.2 per 100,000), Italy (65.4 per 100,000), and the United Kingdom (63.4 per 100,000) are roughly similar to that in the United States (89.2 per 100,000).[22]

An increased risk of breast cancer has been observed among women exposed to radiation from atomic-bomb explosions, repeated chest fluoroscopy, or treatment of postpartum mastitis.[23-28] Most previous studies of large populations of patients who were treated for Hodgkin's disease did not detect a significantly elevated risk of breast cancer.[17,18,29-33] This may be because of the long interval between the occurrence of Hodgkin's disease and the appearance of breast cancer. The paucity of young patients in most reported series must also be taken into account because of the association of the risk of breast cancer with younger age at the time of treatment for Hodgkin's disease.[34] One study of 885 women who were treated for Hodgkin's disease with radiation before 30 years of age found a fourfold increase in the risk of breast cancer.[35] However, only 76 patients in this report were less than 15 years old when Hodgkin's disease was diagnosed; 3 of those 76 patients had breast cancer.

In our study, breast cancer occurred exclusively in women. The majority of breast cancers arose within the field of radiation. We found that the risk of breast cancer increased with the dose of radiation; most breast cancers occurred in patients who had received at least 2000 cGy in the mantle region.

The increased risk of breast cancer after treatment for Hodgkin's disease was related to age at the time of radiation exposure. Sixteen of the 17 breast cancers occurred in patients who were between 10 and 16 years of age when Hodgkin's disease was diagnosed. Hancock et al. reported an increased risk of breast cancer among women who were less than 30 years old when Hodgkin's disease was diagnosed.[35] In atomic-bomb survivors, an increased risk of breast cancer was found in the group of women who were in the first three decades of life when they were exposed to the radiation.[27] The high incidence of breast cancer in women who are exposed to high doses of radiation between 10 and 16 years of age suggests that the tumorigenic influence of radiation mainly affects proliferating breast tissue.

We found that after a relatively short period of latency (4.4 years), the cumulative incidence of leukemia rose sharply, but it appeared to reach a plateau after 14 years, which is consistent with data from other studies.[13] The dose-dependent association of alkylating agents with secondary leukemia and non-Hodgkin's lymphoma has been reported by others.[15,16] The combination of doxorubicin, bleomycin, vinblastine, and dacarbazine appeared to be less leukemogenic than the combination of mechlorethamine, vincristine, procarbazine, and prednisone, but the difference was not statistically significant.

It has not been established that splenectomy is a risk factor for secondary leukemia.[17,36-44] In the original cohort of 979 survivors of Hodgkin's disease in the Late Effects Study Group, splenectomy had borderline significance as a risk factor (P = 0.09),[16] and in the present study, we did not find any independent relation between splenectomy and the risk of secondary leukemia or solid tumors.

In contrast to the risk of treatment-related leukemia, which plateaued after 14 years, the risk of solid tumors continued to increase beyond 15 years and approached 30 percent at 30 years. This is an important problem in survivors of Hodgkin's disease and underscores the ne-
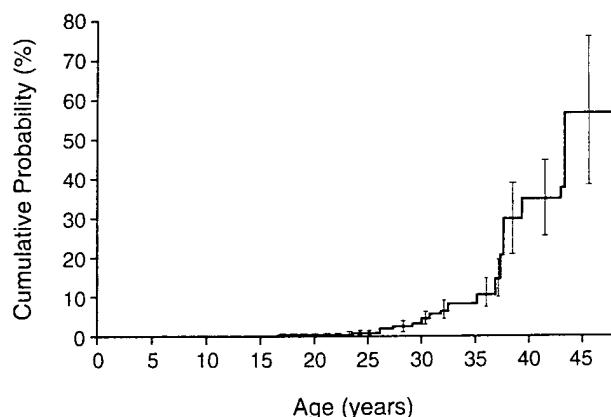
Figure 2. Cumulative Probability of Breast Cancer as a Function of Age in the Cohort of Female Survivors of Hodgkin's Disease in Childhood.

Bars indicate standard errors.

cessity of medical monitoring. The high risk of breast cancer in women exposed to radiation at a young age raises important issues regarding screening programs (such as physical examination of the breast, sonography, mammography, and quantitative magnetic resonance imaging). We must also consider chemoprevention (tamoxifen and retinoids) for survivors of Hodgkin's disease who are at high risk for breast cancer. Efforts to develop treatments for Hodgkin's disease that are curative but less carcinogenic should continue.

APPENDIX

In addition to the authors, the Late Effects Study Group included the following: Dana–Farber Cancer Institute, Boston — S. Sallen and F. Li; Columbus Children's Hospital, Columbus, Ohio — R. Ruymann and W. Newton; Children's Memorial Hospital, Chicago — E. Morgan; Royal Manchester Children's Hospital, Manchester, England — P. Morris-Jones and J. Birch; Emma Kinderziekenhuis, Amsterdam — P.A. Voute; Children's Hospital, Los Angeles — S. Siegel; Children's Hospital Medical Center, Cincinnati — C. DeLaat; Children's National Medical Center, Washington, D.C. — H.S. Nicholson; and Children's Hospital, Pittsburgh — J. Blatt.

REFERENCES

1. Devita VT Jr, Serpick AA, Carbone PP. Combination chemotherapy in the treatment of advanced Hodgkin's disease. Ann Intern Med 1970;73:881-95.
2. Horning SJ, Hoppe RT, Kaplan HS, Rosenberg SA. Female reproductive potential after treatment for Hodgkin's disease. N Engl J Med 1981;304:1377-82.
3. Sherins RJ, DeVita VT Jr. Effect of drug treatment for lymphoma on male reproductive capacity: studies of men in remission after therapy. Ann Intern Med 1973;79:216-20.
4. Rosenberg SA, Kaplan HS. The evolution and summary results of the Stanford randomized clinical trials of the management of Hodgkin's disease: 1962-1984. Int J Radiat Oncol Biol Phys 1985;11:5-22.
5. Arseneau JC, Sponzo RW, Levin DL, et al. Nonlymphomatous malignant tumors complicating Hodgkin's disease: possible association with intensive therapy. N Engl J Med 1972;287:1119-22.
6. Canellos GP, Arseneau JC, DeVita VT, Whang-Peng J, Johnson RE. Second malignancies complicating Hodgkin's disease in remission. Lancet 1975;1:947-9.
7. Coleman CN, Kaplan HS, Cox R, Varghese A, Butterfield P, Rosenberg SA. Leukaemias, non-Hodgkin's lymphomas and solid tumours in patients treated for Hodgkin's disease. Cancer Surv 1982;1:733-44.
8. Coltman CA Jr, Dixon DO. Second malignancies complicating Hodgkin's disease: a Southwest Oncology Group 10-year follow-up. Cancer Treat Rep 1982;66:1023-33.
9. Glicksman AS, Pajak TF, Gottlieb JA, Nissen N, Stutzman L, Cooper MR. Second malignant neoplasms in patients successfully treated for Hodgkin's disease: a Cancer and Leukemia Group B study. Cancer Treat Rep 1982;66:1035-44.
10. Boivin JF, Hutchison GB, Lyden M, Godbold J, Chorosh J, Schottenfeld D. Second primary cancers following treatment of Hodgkin's disease. J Natl Cancer Inst 1984;72:233-41.
11. Koletsky AJ, Bertino JR, Farber LR, et al. Second neoplasms in patients with Hodgkin's disease following combined modality therapy — the Yale experience. J Clin Oncol 1986;4:311-7.
12. Valagussa P, Santoro A, Fossati-Bellani F, Banfi A, Bonadonna G. Second acute leukemia and other malignancies following treatment for Hodgkin's disease. J Clin Oncol 1986;4:830-7.
13. Blayney DW, Longo DL, Young RC, et al. Decreasing risk of leukemia with prolonged follow-up after chemotherapy and radiotherapy for Hodgkin's disease. N Engl J Med 1987;316:710-4.
14. Pederson-Bjergaard J, Specht L, Larsen SO, et al. Risk of therapy-related leukaemia and preleukaemia after Hodgkin's disease: relation to age, cumulative dose of alkylating agents, and time from chemotherapy. Lancet 1987;2:83-8.
15. Tucker MA, Meadows AT, Boice JD Jr, et al. Leukemia after therapy with alkylating agents for childhood cancer. J Natl Cancer Inst 1987;78:459-64.
16. Meadows AT, Obringer AC, Marrero O, et al. Second malignant neoplasms following childhood Hodgkin's disease: treatment and splenectomy as risk factors. Med Pediatr Oncol 1989;17:477-84.
17. Beaty O III, Hudson MM, Greenwald C, et al. Subsequent malignancies in children and adolescents after treatment for Hodgkin's disease. J Clin Oncol 1995;13:603-9.
18. Boivin JF, Hutchison GB, Zauber AG, et al. Incidence of second cancers in patients treated for Hodgkin's disease. J Natl Cancer Inst 1995;87:732-41.
19. Miller BA, Ries LAG, Hankey BF, et al. SEER cancer statistics review: 1973-1990. Bethesda, Md.: National Cancer Institute, 1993. (NIH publication no. 93-2789.)
20. Vandenbroucke JP. A shortcut method for calculating the 95 per cent confidence interval of the standardized mortality ratio. Am J Epidemiol 1982;115:303-4.
21. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958;53:457-81.
22. Parkin DM, Muir CS, Whelan SL, Gao YT, Ferlay J, Powell J, eds. Cancer incidence in five continents. Vol. 6. Lyon, France: International Agency for Research on Cancer, 1992. (IARC scientific publications no. 120.)
23. Mackenzie I. Breast cancer following multiple fluoroscopies. Br J Cancer 1965;19:1-8.
24. Miller AB, Howe GR, Sherman GJ, et al. Mortality from breast cancer after irradiation during fluoroscopic examinations in patients being treated for tuberculosis. N Engl J Med 1989;321:1285-9.
25. Shore RE, Hildreth N, Woodard E, Dvoretsky P, Hempelmann L, Pasternack B. Breast cancer among women given X-ray therapy for acute postpartum mastitis. J Natl Cancer Inst 1986;77:689-96.
26. Baral E, Larsson LE, Mattson B. Breast cancer following irradiation of the breast. Cancer 1977;40:2905-10.
27. Tokunaga M, Land CE, Yamamoto T, et al. Incidence of female breast cancer among atomic bomb survivors: Hiroshima and Nagasaki, 1950-1980. Radiat Res 1987;112:243-72.
28. Boice JD Jr, Land CE, Shore RE, Norman JE, Tokunaga M. Risk of breast cancer following low-dose radiation exposure. Radiology 1979;131:589-97.
29. Cimino G, Papa G, Tura PS, et al. Second primary cancer following Hodgkin's disease: updated results of an Italian multicentric study. J Clin Oncol 1991;9:432-7.
30. Tucker MA, Coleman CN, Cox RS, Varghese A, Rosenberg SA. Risk of second cancers after treatment for Hodgkin's disease. N Engl J Med 1988;318:76-81.
31. Greene MH, Wilson J. Second cancer following lymphatic and hematopoietic cancers in Connecticut, 1935-82. In: Boice JD Jr, Storm HH, Curtis RE, et al., eds. Multiple primary cancers in Connecticut and Denmark. National Cancer Institute monograph 68. Washington, D.C.: Government Printing Office, 1985:191-7. (NIH publication no. 85-2714.)
32. Storm HH, Prener A. Second cancer after lymphatic and hematopoietic cancers in Denmark, 1943-80. In: Boice JD Jr, Storm HH, Curtis RE, et al., eds. Multiple primary cancers in Connecticut and Denmark. National Cancer Institute monograph 68. Washington, D.C.: Government Printing Office, 1985:389-409. (NIH publication no. 85-2714.)
33. Kaldor JM, Day NE, Band P, et al. Second malignancies following testicular cancer, ovarian cancer and Hodgkin's disease: an international collaborative study among cancer registries. Int J Cancer 1987;39:571-85.
34. Curtis RE, Boice JD Jr. Second cancers after radiotherapy for Hodgkin's disease. N Engl J Med 1988;319:244-5.
35. Hancock SL, Tucker MA, Hoppe RT. Breast cancer after treatment of Hodgkin's disease. J Natl Cancer Inst 1993;85:25-31.

36. van Leeuwen FE, Somers R, Hart AAM. Splenectomy in Hodgkin's disease and second leukaemias. Lancet 1987;2:210-1.

37. van der Velden JW, van Putten WLJ, Guinee VF, et al. Subsequent development of acute non-lymphocytic leukemia in patients treated for Hodgkin's disease. Int J Cancer 1988;42:252-5.

38. van Leeuwen FE, Somers R, Taal BG, et al. Increased risk of lung cancer, non-Hodgkin's lymphoma, and leukemia following Hodgkin's disease. J Clin Oncol 1989;7:1046-58.

39. Kaldor JM, Day NE, Clarke EA, et al. Leukemia following Hodgkin's disease. N Engl J Med 1990;322:7-13.

40. Tura S, Fiacchini M, Zinzani PL, Brusamolino E, Gobbi PG. Splenectomy and the increasing risk of second acute leukemia in Hodgkin's disease. J Clin Oncol 1993;11:925-30.

41. van Leeuwen FE, Klokman WJ, Hagenbeek A, et al. Second cancer risk following Hodgkin's disease: a 20 year follow-up study. J Clin Oncol 1994;12:312-25.

42. Andrieu J-M, Ifrah N, Payen C, Fermanian J, Coscas Y, Flandrin G. Increased risk of second acute nonlymphocytic leukemia after extended-field radiation therapy combined with MOPP chemotherapy for Hodgkin's disease. J Clin Oncol 1990;8:1148-54.

43. Abrahamsen JF, Andersen A, Hannisdal E, et al. Second malignancies after treatment of Hodgkin's disease: the influence of treatment, follow-up time, and age. J Clin Oncol 1993;11:255-61.

44. Rodriguez MA, Fuller LM, Zimmerman SO, et al. Hodgkin's disease: study of treatment intensities and incidences of second malignancies. Ann Oncol 1993;4:125-31.